ORIGINAL ARTICLE

PLASMA PROLIDASE ACTIVITY AND ITS RELATION WITH NUMBER OF CYSTS IN POLYCYSTIC OVARY SYNDROME

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Background: Plasma prolidase, a Matrix Metalloproteinase (MMP), has a role in maintaining extracellular matrix remodelling in ovary. The aim of this study was to find out the activity and association with the clinical and biochemical parameters of plasma prolidase in different phenotypes of Polycystic Ovary Syndrome (PCOS). **Methods:** Total 90 women (16–22 years) with PCOS were enrolled in the study. Data regarding anthropometric measurements (weight, height, BMI and WHR) were taken. Transabdominal ultrasonography was done by trained sonographer. Hormonal tests (free testosterone, FSH, LH), and metabolic tests (including FBS) were done by chemiluminescence analyzer. Participants were divided into four groups. Group 1 (n=25), hyperandrogenism with oligo-anovulation (HO), Group 2 (n=26), polycystic ovary with oligo-anovulation (PO), Group 3 (n=20), hyperandrogenism with polycystic ovary (HP), Group 4 (n=19), with presence of all these three features (PHO). Five ml blood sample was taken for plasma Prolidase by Spectrophotometry. Kruskal Wallis test and spearman correlation were used for statistical analysis. **Results:** Among 90 women 52.2% had both polycystic ovaries, 13.3% has one polycystic ovary and 34.4% had normal ovaries on ultrasound. Median difference of plasma Prolidase, LH, FSH, free testosterone and HOMA-IR between groups were found statistically significant (p<0.05). Plasma Prolidase had a direct relation (r=0.374) with number of cysts on ultrasound. **Conclusion:** Plasma Prolidase activity was high in PCOS phenotypes and positive correlation was found between Prolidase levels and number of cysts. Prolidase can be used as diagnostic and prognostic marker in PCOS.

Keywords: Phenotypes, Extracellular matrix, Prolidase, Cysts, Polycystic ovary, Syndrome

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INTRODUCTION

Polycystic Ovary Syndrome (PCOS), a heterogeneous disorder described by hyperandrogenism, oligomenorrhea or amenorrhea, and infertility, affects almost 4–21% of the women of reproductive age.[1–5] In spite of high prevalence of PCOS its diagnosis is still a challenging concern in the field of reproductive medicine.[6] Different criteria have been used for diagnosis of PCOS based on clinical, biological and ultrasonographic findings in which most accepted criteria for PCOS is the Rotterdam ESHRE/ASRM Consensus criteria that requires presence of two out of three hallmark features (hyperandrogenism, oligomenorrhoea, and polycystic ovary).[7] Due to difficulty in evaluating PCOS on both TVS and abdominal ultrasound especially in obese patients, and inconvenience of patients, the diagnosis of PCOS is challenging. The ultrasonography needs expertise in distinguishing follicle number in 3D stroma and ovarian volume accurately. In this way, the syndrome remains undiagnosed and leads to metabolic syndrome and infertility as complication of PCOS.

The PCOS has complex pathophysiology which reflects the interactions between genetic, endocrine and environmental factors.[8,9] Remodelling of extracellular matrix (ECM) of ovary has reported to be one of the newer concepts of PCOS pathophysiology. Remodelling of ovarian ECM is normally required for growth and regression of the follicles during folliculogenesis, ovulation, luteogenesis and luteolysis in association with various endocrinal factors.[10] Matrix Metalloproteinases (MMPs), extracellular proteolytic enzymes are responsible for ECM remodelling.[11] MMPs including collagenase and proteases degrade old collagen and produce imidodipeptides. Prolidase is another MMP that degrades the C terminal of imidodipeptides and releases proline and hydroxyproline, which is utilized for synthesis of new collagen.[12] Increased prolidase activity have also been shown to be accompanying with abnormal ovarian extracellular matrix remodelling.[13,14] It causes follicular atresia leading to increased ovarian stroma, anovulation, and formation of cysts in the ovary.[15] This ovarian ECM remodelling also affects LH and FSH secretion.[16] The polymorphism missense mutation rs267606943 in exon 8 of prolidase gene was found to be one of the causes of PCOS.[11] Proinflammatory cytokines and growth factors are also responsible for raised prolidase activity. Among these factors oxidative stress is main contributor of high Prolidase activity in PCOS.

Due to rapid decline in female fertility caused by PCOS[17] there is need for a biomarker to contribute in the diagnosis and prognosis of PCOS. This study aims to check applicability of prolidase as a biomarker in PCOS.

MATERIAL AND METHODS

This was a cross-sectional study conducted in the Gynaecological OPD at Ziauddin Hospital, Karachi from Jun 2019 to Jun 2020.. The study was approved by Ethic Review Committee of Ziauddin University (ERC Reference No: 0910319MFPHY). All participants were interviewed on an individual basis after taking informed consent. The Rotterdam criterion, established at Rotterdam ESHRE/ASRM Consensus was used to diagnose PCOS.[7] The menstrual irregularity was assessed by the menstrual cycle of less than 21 days or more than 35 days.[18] Ovaries were considered as polycystic on ultrasound by the presence of more than 12 follicles per ovary with diameter 2–9 mm and ovarian size >10 Cm.[19] Hyperandrogenism was evaluated clinically and biochemically. Five ml venous blood was drawn. Plasma Prolidase activity was assayed using method defined by Bhatnager *et al*[19]. Blood plasma was incubated with Manganese (Mn^{+2}) for the activation of plasma Prolidase by mixing 100 µL of 20 mmol/L $MnCl_2$ and 100 µL of 0.05 mol/L Tris HCl (*p*H 7.8) in 100 µL of plasma, monitored by incubation at 37 °C for 1 hour. Then 100 µL of 94 mmol/L glycine-proline solution with 100 µL of incubated solution was mixed and mixture was again incubated for 1 hour at 37 °C. After 1 hour, 1 mL of 0.45 mol/L trichloroacetic acid (TCA) was added to stop further reaction. To 0.5 mL of supernatant, 2 mL of 1:1 mixture of chinard reagent:glacial acetic acid was added following incubation of 10 min at 90 °C. Absorbance was taken at 515 nm in spectrometer and by using proline standard Prolidase activity was calculated. Activity was described as the quantity of Prolidase required to convert 1 µmol substrate in 1 min. The results are expressed in units per litre (U/L).

PCOS patients were divided on the basis of Rotterdam criteria into following four phenotypes:

Group 1: Hyperandrogenism+Oligo-anovulation (HO) n=25
Group 2: Polycystic ovary+Oligo-anovulation (PO) n=26
Group 3: Hyperandrogenism+Polycystic ovary (HP) n=20
Group 4: Presence of all three feature (PHO) n=19

Data was analysed using SPSS-22. Quantitative variables were presented as median and interquartile ranges. Qualitative variables were presented as frequency and percentages. Kruskal Wallis test was used to find association between groups. Spearman correlation was applied to find association between variables, and $p<0.05$ was considered statistically significant.

RESULTS

Majority of the women (52.2%) had both polycystic ovaries, 13.3% had one polycystic ovary and 34.4% had normal ovaries in ultrasound.

Table-1 indicates comparison among the four phenotypes of PCOS. The median Prolidase activity was also significantly ($p<0.04$) higher among patients with PHO 800 IU/L, when compared with HO, OP, and PH groups. The median value of FSH was significantly ($p<0.00$) higher in HO group, 6.7 mIU/mL when compared with OP, PH and PHO. Similarly, the median LH level was also significantly ($p<0.02$) higher among patients with PHO 17.5. Regarding the median free testosterone level was significantly ($p<0.01$) higher among patients with HO group, 110 ng/dL when compared with OP, PH and PHO. The median insulin level was also significantly ($p<0.001$) higher among patients with PH 22.6 mIU/L HO group, 12 mIU/L when compared with OP, and PHO. However, no significant difference found for FBS, WHR, FBS TSH, prolactin between the groups.

Table-2 shows there was significant increase in no of cysts ($p<0.001$) in PHO group seen as compared to HO, OP and HP groups. Significantly increased median stroma of right ovary ($p<0.001$) found in PHO group than that of HO, OP and HP groups. Significant increased stroma of left ovary ($p<0.001$) found in PHO group than that of HO, OP and HP groups. Hence result showed PHO group had statistically significant increased no of cysts and stroma of right and left ovary while HO group has decreased no of cysts and stroma of right and left ovary.

In correlation of plasma prolidase activity with metabolic and hormonal parameters our result showed positive correlation of number of cysts ($p<0.001$) and free testosterone ($p=0.03$) as shown in Figure-1 and Figure-2 while negative correlation of FSH ($p=0.006$) observed with plasma prolidase activity. Though there was no correlation of BMI, FBS, LH, prolactin, TSH, insulin, and HOMA IR with plasma prolidase activity.

Tabel-1: Comparison of biochemical and hormonal parameters among PCOS groups

Parameters	HO		OP		PH		PHO		*p*
	M	IQR	M	IQR	M	IQR	M	IQR	
Fasting Blood Sugar (mg/dL)	100.0	10.0	96.5	10.0	97.0	9.5	95.0	10.0	0.43
LH (mIU/mL)	12.60	5.33	13.54	7.7	15.4	5.9	17.5	2.4	0.02*
TSH (mIU/L)	2.4	1.8	2.6	1.9	2.3	1.7	2.9	1.5	0.56
Follicular Stimulating Hormone (mIU/mL)	6.7	3.03	6.2	2.7	6.5	2.9	1.9	0.1	0.00*
Free testosterone (ng/dL)	110.0	30.2	38.5	28.3	92.6	30.8	97.2	17.5	<0.01*
Prolactin (ng/mL)	12.6	9.0	11.4	4.5	9.5	10.4	12.0	9.0	0.36
Insulin (mIU/L)	12	6.1	19.2	6.2	22.6	8.26	21.5	9.0	<0.001*
Prolidase (IU/L)	611.8	147.0	690.0	111.89	721.95	118.7	800.0	173.20	0.04*

M=Median, IQR=Interquartile range, *Significant

Table-2: Comparison of ultrasound findings in groups of PCOS

Parameters	HO		OP		PH		PHO		*p*
	M	IQR	M	IQR	M	IQR	M	IQR	
Number of cysts	0.0	0	22.0	8.75	21.5	2.5	26.0	2.25	<0.001*
Stroma of right ovary (Cm)	6	2.0	11.0	3.5	10.3	5	11.05	3.25	<0.001*
Stroma of left ovary (Cm)	6	1.0	10.3	7.4	9.2	7	12	4.25	<0.001*

M=Median, IQR=Interquartile range, *Significant

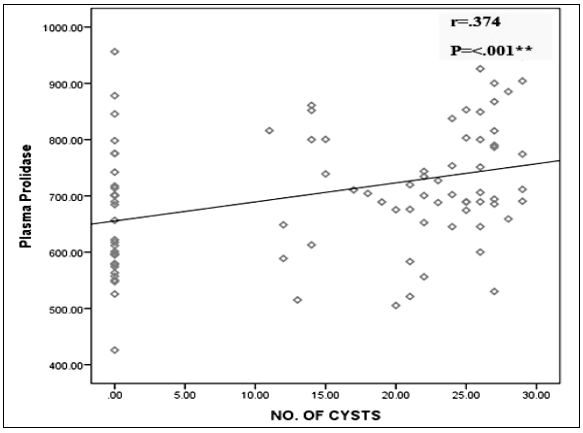

Figure-1: Scatter plot between plasma prolidase and Number of cysts

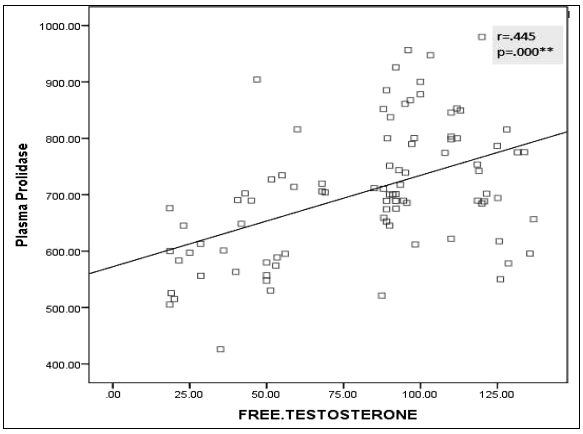

Figure-2: Scatter plot between plasma prolidase and free testosterone

DISCUSSION

The present study was done to find out the role of plasma prolidase in pathophysiology of PCOS. Plasma Prolidase activity was found to be significantly high in PCOS patients specifically in PHO group suggesting severity of the syndrome. These findings have also been reported in other studies conducted on patients with PCOS.[14,15,19–21] We observed positive correlation of Prolidase activity with the number of cysts which corroborates with previous work[15,19]. Increased Prolidase activity may be the cause of formation of cysts and an ovulating antral follicle in the ovaries due to abnormal remodelling of ECM.[19] The association of plasma Prolidase activity with PCOS phenotypes and number of cysts has highlighted its significance as a prognostic marker during its management.

Our study showed most of the PCOS patient with the bilateral polycystic ovaries on ultrasound while Bhatnager *et al*[19] found equal number of normal and polycystic ovaries, among PCOS patients. In our study number of cysts, stroma of left and right ovary significantly increased in group PHO. This might be due to high levels of free testosterone and androgens which causes proliferation of stromal cells and theca cells of ovaries and ultimate cause cyst formation. Alviggi *et al*[22] also reported increase in stroma of ovaries in PCOS.

We observed increased levels of free testosterone, FSH and LH in PCOS groups. Highest level of free testosterone was found in OH group while highest levels of LH and lowest level of FSH were seen in PHO group. This may be due to the fact that PCOS is a consequence of abnormal inter-related endocrinal changes.[23] A study conducted in India[24], observed that median testosterone levels were significantly high among different phenotypes with highest testosterone level in PHO phenotype while no difference in LH was detected in different phenotype groups. Wiweko *et al*[25] reported high serum LH levels in phenotype PHO, with no differences in FSH level. Our study has reported different findings of hormonal changes as compared to other studies which may be due to racial or ethnicity differences. This study has reported positive correlation of prolidase activity with free testosterone. Increase in androgens causes stromal cell proliferation that causes abnormal ECM remodelling and high prolidase activity. Neoklis AG *et al*[26] found association of classical phenotypes of PCO, having all three features of PCOS, with deranged LH/FSH ratio, hyperandrogenemia in patients of PCOS with normal BMI.

We observed high levels of FBS among the PCOS groups. Significantly high insulin and HOMA-IR levels were found among the groups with the highest values in PH group. Amini *et al*[27] reported highest mean fasting insulin and HOMA-IR among cases with phenotype PHO while high FBS was found in PH group. It has also been reported that the down regulation of intracellular antioxidant systems as well as increased production of ROS in the adipose tissue in PCOS causes oxidative stress leading to development of IR.[28] IR cause compensatory hyperinsulinemia in PCOS which in turn interacts synergistically with LH, as a co-gonadotropin within ovarian theca cells.[29] It sends signals to the ovaries to increase androgen production by activation of CYP17 (encoding P450c17α), a key enzyme in ovarian androgen biosynthesis.[30]

This study has highlighted increased plasma prolidase activity as a possible molecular cause of PCOS. Its significant correlation with number of cysts will help in management of PCOS to prevent its metabolic complications and infertility in PCOS.

There are a few limitations to our study. It is a single-centred, cross-sectional study. There is need to perform prolidase activity on a large sample size from multiple centres. A case control study design is required to establish prolidase as a diagnostic biomarker and follow-up studies with the correlation of number of cysts should be done monitoring disease progress and reproductive outcomes.

CONCLUSION

Plasma prolidase activity was high in PCOS thereby confirming its role in pathophysiology of PCOS. Hence it can be used as a diagnostic biomarker in PCOS. There is a positive correlation found between prolidase activity and number of cysts. This makes it as a prognostic marker to monitor number of cysts or disease status. Future studies, with bigger sample size, are required to establish plasma prolidase activity as potential biomarker for PCOS.

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MF: Original idea, sample collection, experimentation and initial writing
SA: Article writing and sample processing
MII: Literature review and article writing
STA: Reference, critical analysis and logical reasoning
HA: Grammar, and data analysis
RR: Proofreading, Critical analysis

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